Transition of Endothelium to Cartilage and Bone

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Mesenchymal stromal cells (MSCs) are capable of differentiating into bone-forming osteoblasts. A recent *Nature Medicine* study (Medici et al., 2010) shows that the mislocalized bone in the human disease fibrodisplasia ossificans progressiva (FOP) originates from vascular endothelium that gives rise to MSCs.

Ectopic bone formation in soft tissues is a common occurrence following trauma, internal muscular bleeding, osteoarthritis (OA), inflammation, and also in specific genetic disorders. One such condition is fibrodisplasia ossificans progressiva (FOP), in which cartilage and bone form pathologically within soft tissues rather than only within the skeleton. Olsen and colleagues studied the source of ectopic bone in individuals inflicted with FOP (Medici et al., 2010). Mesenchymal stromal cells (MSCs) are multipotent cells with bone-, fat-, and cartilage-forming potential that are widespread in calcified and soft tissues and have been presumed to be the source of mislocalized bone. In FOP, heterotopic ossification is thought to occur through mesenchymal condensation, followed by chondrogeneis, and finally endochondral ossification. Olsen and colleagues show that vascular endothelial cells that undergo endothelial-to-mesenchymal transition (EndMT) are the source of cells that generate cartilage and bone lesions (Medici et al., 2010). This phenomenon of transdifferentiation of endothelium into bone, as demonstrated in the FOP model, shows that the human disease recapitulates hallmarks of embryonic plasticity. The ability of FOP-derived endothelial cells to undergo EndMT is related to a mutation in the receptor ALK2, which causes its constitutive activation. This observation leaves open the possibility that the unmutated form of ALK2 might not mediate EndMT. However, the authors also demonstrate that activation of endothelial cells with ALK2 ligands, such as transforming growth factor (TGF)-β superfamily cytokines (Figure 1), results in the transition of endothelium into mesenchyme. Therefore, EndMT may be a physiological occurrence, and not necessarily restricted to a diseased state.

The Olson et al. study makes a strong case that EndMT provides a mechanism for heterotopic bone formation, based, in part, on their analysis of diseased tissues. Both humans with FOP and mice with mutated ALK2 develop heterotopic bone, the phenotype of which includes expression of relevant cartilage and bone markers, as well as the endothelial markers TIE2 and vWF. These observations are substantiated through the use of reporter mice that express an enhanced green fluorescence protein (EGFP) transgene under the control of the endothelial-specific Tie2 promoter. Analysis of EGFP expression in sections of ligand-induced heterotopic cartilage and bone revealed that many green endothelial-derived cells are also Sox9 (cartilage) and osteocalcin (bone) positive (Medici et al., 2010). The hybrid endothelial/mesenchymal phenotype observed in vivo suggests that mutant ALK2 mediates the transition from endothelium to cartilage and bone, and results from subsequent culture experiments support this hypothesis. Specifically, expression of the mutant ALK2 in human cultured endothelial cells (HUCEC) and in human cutaneous microvascular endothelial cells (HCMEC) resulted in the acquisition of fibroblast morphology, associated with the expression of classical markers of epithelial-to-mesenchymal transition (EMT), including Snail and Slug. The transition of endothelium into mesenchyme is also supported by the appearance of the fibroblast marker FSP-1 in early lesions of the mutant mice induced with the ALK2 ligand, bone morphogenic protein (BMP)-4. In both in vitro experiments and an in vivo immunocompromized mouse model, the mutant ALK2 expressing endothelial cells gave rise to osteogenic, adipogenic, and chondrogenic mesodermal lineages, consistent with the proposal that the endothelial cells dedifferentiated into MSCs. This pathway, involving the acquisition of MSC phenotype and function by endothelium, is not dependent on the presence of the constitutively active, mutant ALK2. Indeed, endothelial cells exposed to the ALK2 ligands TGF-β2 and BMP4 also differentiated, both in vitro and in vivo, into the aforementioned three mesodermal lineages. Finally, because the knockdown of this receptor prevented the transition, the study provides evidence that EndMT in this system is dependent on signals downstream of ALK2.

The combination of in vivo observations, in vitro findings, and the analysis of the molecular mechanism of EndMT (Medici et al., 2010) constitute a solid study that demonstrates an alternate pathway of chondrogenesis and osteogenesis. One caveat to the findings presented by Olsen and colleagues that will require further investigation relates to the current dependence on the expression of specific cell markers. Surface phenotype determination may not always identify cell lineages faithfully. Further analysis that establishes specific endothelial function is required in order to complement the existing assessment of functional mesenchymal traits, namely, multilineage differentiation potential. Future studies should also explore the possibility that other cases of ectopic ossification might be due to EndMT. In osteoarthritis (OA), as one example, ectopoic ossification causes severe pain and disability. The mechanism of OA is not well understood, and elucidation of the possible contribution of the microvasculature is now necessary. Futhermore, EndMT may not be

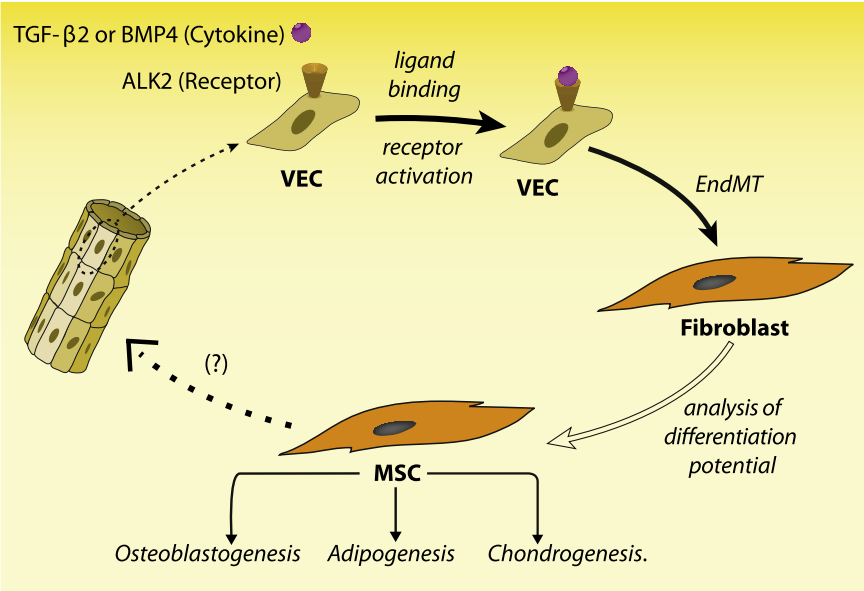

Figure 1. A Putative Cycle of Cell-Fate Transitions
Vascular endothelium activated by appropriate ALK2 ligands, such as TGF-β2, undergoes an endothelial-mesenchymal transition (EndMT), leading to acquisition of fibroblast morphology and markers, and multipotency that defines mesenchymal stromal cells (MSCs). Multipotency is demonstrated by the ability of the cells produced by EndMT to differentiate, upon specific induction, into osteoblasts, adipocytes, and chondrocytes. The reported potential of MSCs to differentiate into endothelial cells completes the putative cycle. The question mark indicates that this portion of the cycle has not been demonstrated in the present study.

restricted to pathological conditions, and bone remodeling and fracture repair may entail similar processes in which the vasculature serves as the source of osteogenic cells. In addition, it is tempting to speculate that EndMT may represent a physiological mechanism for the generation of MSCs. Perivascular cells, specifically pericytes (Crisan et al., 2008), have been suggested to be the in vivo counterparts of cultured MSCs. The present study provides evidence that the endothelium itself serves as an alternative source.

The observation of EndMT in adult tissues, albeit diseased, reawakens the debate as to the plasticity of cell behavior in the adult. Studies published almost 10 years ago proposed that adult hematopoietic stem cells, adult MSCs, and a variety of tissue-specific progenitors can undergo transdifferentiation. For example, Sharkis and colleagues published that bone-marrow-derived cells could produce mature cells of epithelial organs, such as the liver and lung (Krause et al., 2001). Other examples of transitions from one fully differentiated cell type into mature cells of a different lineage/tissue have been reported and were suggested to entail dedifferentiation. The present report by Olsen et al. can be added to the list of studies supporting the notion of cellular plasticity in adult mammalian tissues. Notably, this report is not isolated. Several other recent studies also support the possibility that cellular plasticity is neither restricted to the embryo nor to diseased adult tissues. Studies of mouse and human spermatogonia highlight the fact that these cells are easily reprogrammable under mild conditions (Conrad et al., 2008), which do not require the use of harsh genetic manipulations. Even more striking is the finding that the dedifferentiation of maturing germ cells back into spermatogonial stem cells occurs under stress (Nakagawa et al., 2007), and even spontaneously and frequently (Klein et al., 2010), supporting the model that dedifferentiation is a physiological phenomenon. An example of mammalian dedifferentiation and transdifferentiation has also been recently observed in the pancreas (Thorel et al., 2010).

A fraction of the MSC population constitutes multipotent cells that give rise to a variety of cell types, including endothelium (Conrad et al., 2009). Thus, a complete cycle may exist in which EndMT leads to the formation of MSCs, which, in turn, differentiate back into endothelium through a mesenchymal-to-endothelial transition (MEndT) (Figure 1). This reversibility in cell-fate determination has been used to propose the model of a "stem state" (Zipori, 2004), in which stemness is considered a transient state in a cell's life cycle. In other words, cells may differentiate, but this change does not determine their status permanently. Upon demand for tissue repair, cells downstream in the differentiation cascade may "turn back" and re-exhibit stemness by regaining additional lineage potentials that had previously been lost. The stem state notion predicts that dedifferentiation is possible in mammalian tissues (Zipori, 2009), and this proposal is supported by the current findings that supposedly unipotent adult endothelium can, when prompted, re-exhibit multipotency.

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